LIFE HISTORY AND HABITS OF TWO NEW NEMATODES PARASITIC ON INSECTS[1]

[PRELIMINARY PAPER]

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INTRODUCTION

While investigating the life history and methods of control of the elm borer (*Saperda tridentata* Oliv.) and the termite (*Leucotermes lucifugus* Rossi) at the Kansas Agricultural Experiment Station, two new nematodes were found, one parasitic on the former and the other parasitic on the latter. One hundred and twenty-one adult beetles obtained from one tree[2] were placed in breeding cages, but in no instance were eggs deposited, and both sexes eventually weakened and died. Examination after death showed that the intestines were so filled with nematodes that in only one female were eggs even developed in the body. The death rate due to nematode parasitization was apparently 100 per cent. Several colonies of *Leucotermes lucifugus* were placed in salve boxes, together with food. Inasmuch as *Saperda tridentata* had shown so high a nematode parasitization, it was naturally suggested that nematodes might be present in the termites. Accordingly a number of these insects were killed and examined, with the result that nematodes were found infesting the head in varying degrees. Of the colonies taken, 76.92 per cent were parasitized with nematodes. The parasitism of the individuals in single colonies ranged from 0 to 100 per cent.

DIPLOGASTER LABIATA

The nematodes were submitted to Dr. N. A. Cobb, of the Bureau of Plant Industry, United States Department of Agriculture, for identification. He found that the nematode parasitizing *Saperda tridentata* was a new species which he named "*Diplogaster labiata*" (fig. 1; 2, *A–H*), and described as follows:

Diplogaster labiata, n. sp. $\frac{1.2\ \ 17.\ \ 21.\ \ '59'^{48}\ \ 91.}{2.1\ \ 4.2\ \ 4.2\ \ 4.4\ \ 2.9}$ 0.66 mm. (The formula was derived from a single specimen.) The thin layers of the transparent, colorless, naked cuticle are traversed by fine transverse striæ, resolvable with high powers into rows of dots, more particularly near the head and on the tail, those on the tail being somewhat irregularly placed. The cuticle is also longitudinally striated, and the dots of the transverse striations are coincident with those of the longitudinal striations. The longi-

[1] Contribution from the Entomological Laboratory, Kansas State Agricultural College, No. 17. This paper embodies the results of some of the investigations undertaken by the authors in the prosecution of projects Nos. 13 and 101, Kansas Agricultural Experiment Station.

[2] A tent was placed around an elm tree so that all emerging insects might be secured for breeding purposes.

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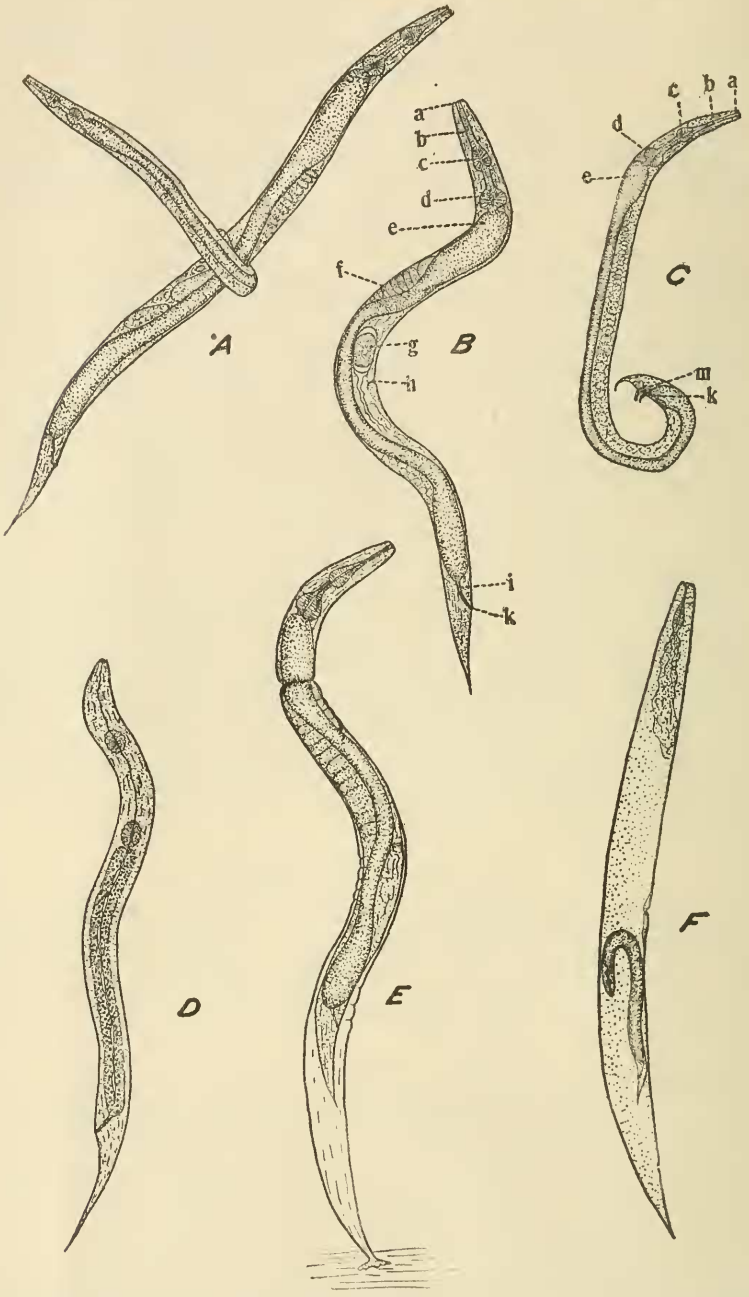

FIG. 1.—*Diplogaster labiata:* *A*, Mating (× 125); *B*, mature female reared in water culture (× 125), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *f*, ovaries, *g*, egg, *h*, genital pore, *i*, rectum, *k*, anus; *C*, mature male reared in water culture (× 125), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *k*, anus, *m*, spicula; *D*, at time of hatching (× 400); *E*, female during process of molting (× 125); *F*, dead female with young nematode which hatched within her body (× 125). Drawings by A. L. Ford.

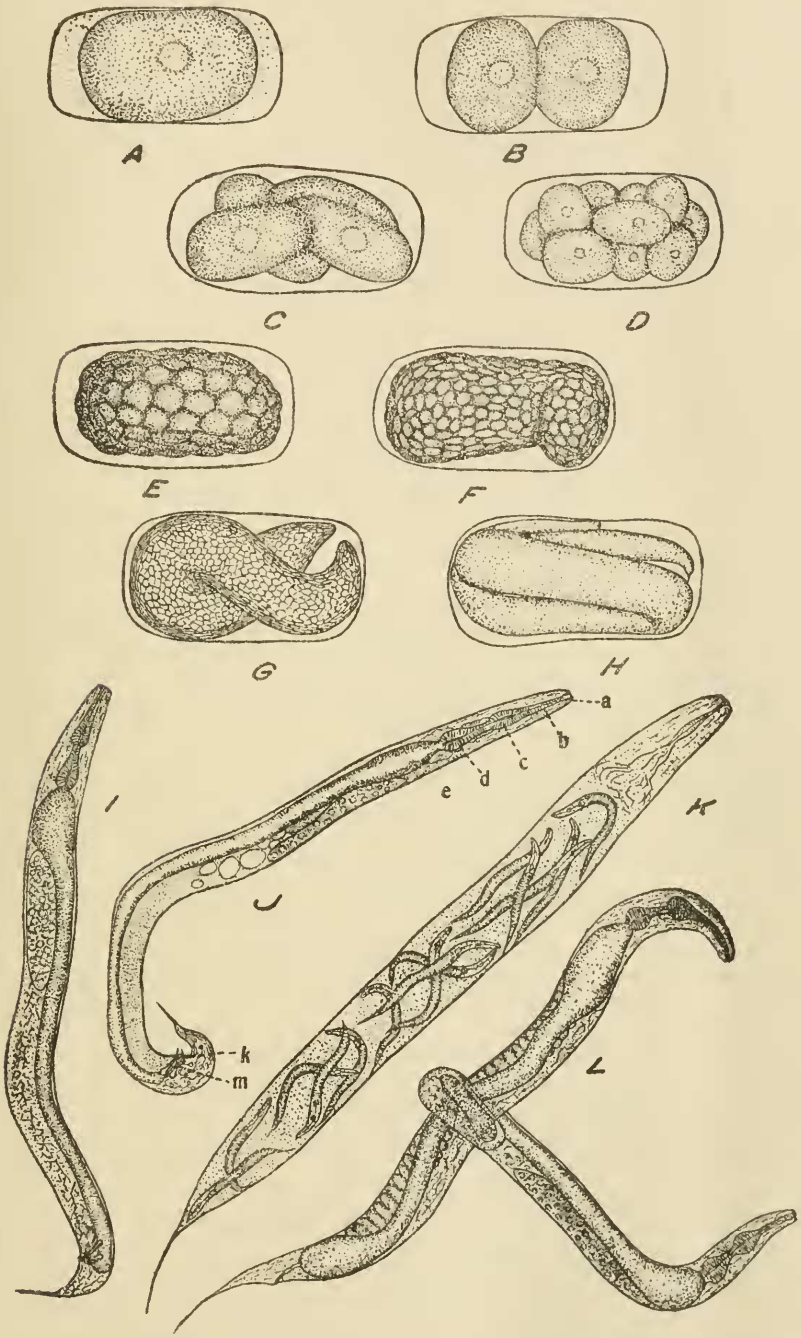

FIG. 2.—*A–H, Diplogaster labiata:* Development of the egg (× 500); *I*, Diplogaster aerivora: mature male reared in moist soil (× 160); *J, Diplogaster aerivora:* mature male reared in water culture (× 125), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *k*, anus, *m*, spicula; *K, Diplogaster aerivora:* dead female with young which hatched within her body (× 125); *L, Diplogaster aerivora:* mating (× 125). Drawings by A. L. Ford.

tudinal striæ are not present on the lateral fields, this naked space being one-third to one-half the width of the body. The slightly conoid neck becomes slightly convex-conoid near the head, the lip region of which is set off by a very broad, almost imperceptible constriction. There are six strongly developed and fairly distinct lips, each ending in a conoid tip, from the summit of which issues a very short innervated bristle-like papilla. The lips have a more or less distinct refractive framework and are in all probability quite mobile. Usually in specimens which have been fixed in Flemming's solution the tips of the lips are slightly outward-pointing, leaving a somewhat circular refractive mouth opening about two-fifths as wide as the front of the head. The inner surface of the lips is so strongly refractive that usually the posterior limits of the lips are distinctly visible, more particularly as the wall of the pharynx at this point is encircled by a very delicate refractive line lying considerably in front of the middle of the pharynx. This latter appears to be irregularly cylindroid, but is slightly unsymmetrical at the base. On the whole, it is about two-fifths as wide as the head. It appears to possess at the base a rather well-developed but blunt, slightly inward-projecting process or tooth. In the lateral view, as the posterior part of the pharynx appears to pass around this projection, it acquires the slightly unsymmetrical contour already mentioned. The walls of the esophagus are rather distinctly ceratinized. The esophagus begins at the base of the pharynx as a tube two-thirds as wide as the base of the head and continues to have this diameter, or a slightly greater, until it reaches a point halfway back to the median bulb. Thence onward it diminishes slightly, so that just in front of the median bulb it is only half as wide as the middle of the neck. The median bulb is a well-developed, elongated or ellipsoidal, radially muscular structure, with a somewhat distinct elongated but narrow valve. This bulb is about two-thirds as wide as the middle of the neck. Behind the median bulb the esophageal tube continues with a diameter one-third to two-fifths as great as the corresponding portion of the neck but diminishes very slightly, so that just in front of the ellipsoidal cardiac bulb it is less than one-third as wide as the corresponding portion of the neck. The cardiac bulb contains a rather distinct and rather complicated threefold valvular apparatus and is capable of opening out posteriorly, so that the lumen of the posterior part of the bulb, where it debouches into the intestine, then becomes one-fourth as wide as the corresponding portion of the body. The lining of the esophagus is a distinct feature throughout its length. The intestine, which is thin-walled at first, is separated from the esophagus by a distinct constriction. It becomes at once four-fifths to five-sixths as wide as the body and presents at the beginning a distinct cardiac cavity. There is also a distinct cardia. The cells of the intestine, which are of such size that probably four are required to build a circumference, contain rather large nuclei and are packed with granules of variable size, the largest of which have a diameter as great as the distance between two of the longitudinal striæ, the smallest of which are very much smaller. The lining of the intestine is refractive, so that the lumen is usually quite a distinct feature. From the slightly raised anus the narrow, refractive, ceratinized rectum, which is one and one-half to two times as long as the anal body diameter, extends inward and forward. The tail end begins to taper from some distance in front of the anus but in front of the anus tapers only very slightly. Behind the anus it tapers rather regularly to an acute point. Near the middle of the tail there appears to be a lateral papilla on each side. From the slightly raised, rather broad vulva the vagina leads inward at right angles to the ventral surface nearly halfway across the body, where it joins the two uteri, which extend in opposite directions. The reflexed ovaries reach more than halfway back to the vulva, at any rate in apparently young specimens in which no eggs exist in the uterus. The ova in the ovary are arranged more or less single file for about half its length; toward the blind end they are arranged irregularly. Fertilized females show sperm cells in the uterus of such a

size that about four to five side by side would span the body diameter. Numerous micro-organisms were seen in the intestine.

Male formula. $\frac{1.9\ \ 16.\ \ 21.\ \ {}^{1}M^{159}.\ \ 94.}{1.7\ \ 3.1\ \ 3.5\ \ 3.9\ \ 2.9}$ 0.72 mm. (single specimen). The tail of the male differs materially in form from that of the female. It begins to taper at the anus, and it tapers rapidly in the anterior two-thirds, more particularly in the middle third, so that at the beginning of the final third it is only about one-tenth as wide as at the anus. Thence onward it tapers rather regularly to the exceedingly fine terminus; there is, however, a pronounced ventral elevation at the beginning of the small part of the tail, though it remains uncertain whether this elevation is innervated. The middle portion of the tail is strongly convex-conoid, the convexity existing largely on the dorsal side. The cuticle of the tail presents a peculiar arrangement of the dots, such that there is an appearance of two sets of oblique fibers crossing each other, these fibers being arranged approximately at 45° to the longitudinal lines. The two equal, rather uniform, somewhat arcuate, blunt spicula are about one and one-fourth to one and one-half times as long as the anal body diameter. Their proximal ends, which are slightly narrower than the main portion, are set off by a rather broad and prominent constriction. At their widest part, through the middle, they are about one-fifth to one-sixth as wide as the corresponding portion of the body. The accessory piece is about half as long as the spicula. It is very inconspicuous near the anus, but lies parallel to the spicula. It widens out to a somewhat clavate or elongated pyriform contour, and has its rounded proximal end toward the dorsal side of the body, and from this blunt end muscular fibers pass obliquely backward to the ventral surface of the tail and join the caudal wall at a distance nearly half way from the anus to the beginning of the narrow portion. Oblique copulatory muscles are to be seen opposite the ejaculatory duct for a distance about one and one-half times as great as the length of the tail. The male papillæ are arranged as follows: One ventrally submedian pair a little in front of the proximal ends of the spicula; one ventrally submedian pair a little in front of the anus, and one ventrally sublateral pair on the same zone; another sublateral pair just opposite the anus; a lateral pair slightly behind the middle of the enlarged portion of the tail; a submedian pair nearly halfway from that last mentioned to the beginning of the small part of the tail; a dorsally sublateral pair a little in front of the beginning of the narrow portion of the tail; three subventral pairs close together opposite that last mentioned; between the members of these three subventral pairs, possibly a single ventral papilla. The most pronounced of these papillæ can hardly be called digitate. The ejaculatory duct is about two-fifths as wide as the body. The vas deferens is nearly two-thirds as wide as the body. The testis tapers so that at the point of inflection, a short distance behind the cardiac bulb, it is about one-fourth as wide as the body. The blind end lies about two body widths behind the flexure.

Habitat: Manhattan, Kans., 1915, on *Saperda tridentata*.

The eggs of *Diplogaster labiata*, elliptical in shape, about twice as long as wide, with bluntly rounded ends, when freshly deposited, were uniformly dark brown or gray, but after segmentation began they became darker. Their average length was 0.0627 mm. and the average diameter 0.031 mm. They were laid singly with apparently no preference as to the place of deposition. Occasionally segmentation began before the eggs were deposited. From the beginning of segmentation the cell divisions could be plainly followed throughout (fig. 2, *A–H*).

A few hours before emerging, the folded young nematodes made slight movements within the egg. Later these movements became

more vigorous until finally they ruptured the shells and emerged, after which the egg walls collapsed. Occasionally a young nematode hatched within the body of a dead female. In cultures the eggs hatched in from 30 to 32 hours from the time of deposition, and the nematodes matured in from 7 to 10 days. The males appeared to mature slightly in advance of the females.

At hatching, the young nematodes were about 0.2 mm. in length (fig. 1, *D*), very slender, and sluggish, and remained for a time in a curled position. Later they straightened out their bodies and became very active. The young worms were almost transparent (in water cultures), there being no solid food in the alimentary canal. As development proceeded, the young became darker in color and more active. At the end of 5 days the sex organs began to appear, and in from 7 to 10 days the nematodes reached maturity.

Specimens which were isolated and kept under observation were noted to molt at least three times, these molts occurring about three days apart. The process of molting (fig. 1, *E*) was as follows: The nematode first fastened its posterior end to any surface upon which it might be resting. The skin then broke at the anterior end and the nematode began to emerge. At first the process was very slow, owing to the fact that the opening of the molt skin was smaller in diameter than the middle part of the body. By moving vigorously from side to side, the nematode slowly worked its way out of the skin. After the widest portion of the body had passed through the opening, no further resistance to emergence was offered, as the posterior end rapidly decreased in diameter. The nematodes were not always able to emerge, as occasionally specimens were found which died before completing the process. Molting lasted from 45 minutes to 6 hours.

The adults and the young were similar in form and food habits, but differed in that the adults possessed sex organs. The mature females were about 0.7 mm. in length and 0.03 mm. in diameter, while the males were about 0.6 mm. in length and 0.02 mm. in diameter.

As soon as maturity was reached, mating began (fig. 1, *A*). The male fastened its caudal end around the middle of the female's body. During this process the male held its body rigid, while the female moved vigorously from side to side. It was not uncommon to find males in the act of mating with their bodies wrapped twice about the females. Toward the end of the process the female increased her activity and soon shook the male free. Many matings were observed, the shortest of which lasted about 2 minutes and the longest 30 minutes.

Proportion of sexes.—Of 367 specimens examined, 229 were found to be females and 138 were males. In other cultures in which counts were not made the females were noticed to be more abundant than the males.

PERIOD OF OVIPOSITION.—While in the specimens of *Diplogaster labiata* under observation mating usually occurred but once, occasionally a few individuals mated a second time. Oviposition began from two to four hours after mating and lasted over a period of about two days, during which time the average number of eggs deposited was seven.

HABITS.—These nematodes infested the intestines of adults of *Saperda tridentata* in such large numbers that they prevented these insects from performing their natural functions. They lived in the alimentary canal in such large numbers that they ruptured the walls of the canal and, escaping into the body cavity of the insect, caused its death.

The examination of individuals of *Saperda tridentata* which had died in this manner rarely showed eggs that had started to develop. Specimens of *Diplogaster labiata* placed in water cultures were fed on macerated bodies of *Saperda tridentata*. They flourished on this, but since the supply was soon exhausted, substitute foods had to be used. Different substances were tried with varying success, but macerated beetles placed in water seemed to be the most satisfactory. Nematodes in cultures without food usually did not live longer than two days. The presence of food acted as a stimulant to copulation and oviposition, but both varied directly with the abundance and adaptability of the food.

The nematodes seemed to show no preference to either day or night for depositing their eggs or any other of their habits.

LENGTH OF ACTIVE BREEDING STATE.—If the nematode is considered to be mature from the time of mating, it spends an average of about two days as a normal active breeding adult.

DIPLOGASTER AERIVORA

In 1856, Charles Lespés[1] gave a meager description of a nematode which he found parasitizing *Leucotermes lucifugus*. His description is short and so indefinite that it might apply to several species of nematodes, but the habits he discusses closely resemble those of the nematodes found in *L. lucifugus* in Kansas. However, Dr. Cobb identified this nematode as *Diplogaster aerivora* (fig. 2, *I–L;* 3) and described it as follows:

Diplogaster aerivora, n. sp. $\frac{.8\ \ 8.9\ \ 12.\ \ '51^{162}\ \ 87.}{1.6\ \ 3.9\ \ 4.9\ \ 5.9\ \ 2.6}$ 1.5 mm. The transparent, moderately thin layers of the colorless naked cuticle are traversed by fine transverse striæ, resolvable with high powers under favorable conditions. The cuticle is traversed also by 24 longitudinal striæ. These longitudinal striæ are sometimes resolvable into quadrate elements, each consisting of four punctations arranged in a quadrangle whose width is equal to the width of the stria. In the majority of specimens these quadrate elements were not to be seen. The distance between the striæ varies in different parts of the body up to about twice their width. The striations of the cuticle, both transverse and longitudinal, vary within pretty wide limits, the varying

[1] Lespés, Charles. Sur un nématoïde parasite des Termites. *In* Ann. Sci. Nat. Zool., s. 4, t. 5, p. 335–356. 1856.

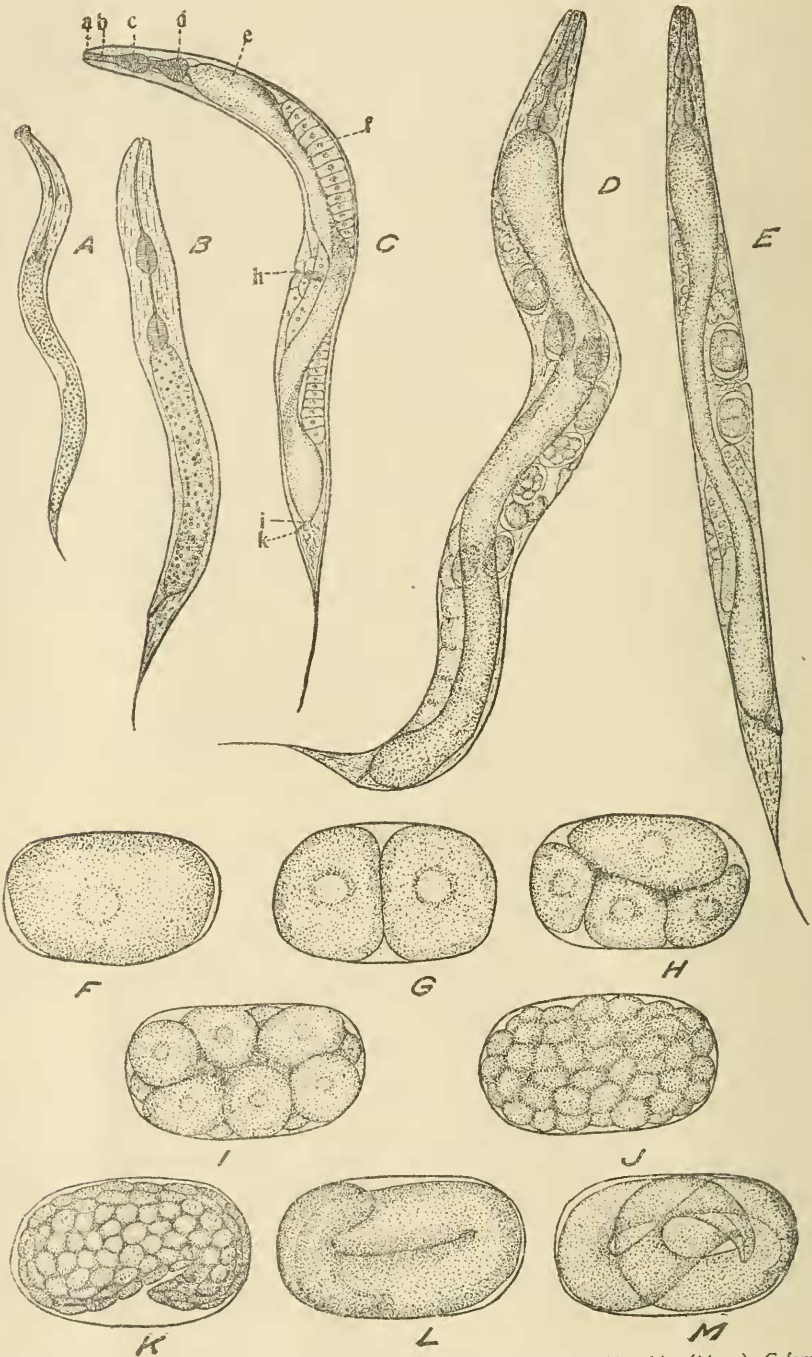

FIG. 3.—*Diplogaster aerivora:* *A*, Form found in termite (× 150); *B*, at time of hatching (× 400); *C*, female reared in water culture, not quite mature (× 100), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *f*, ovaries, *h*, genital pore, *i*, rectum, *k*, anus; *D*, mature female reared in moist soil (× 75); *E*, mature female reared in water culture (× 125); *F–M*, development of the egg (× 500). Drawings by A. L. Ford.

conditions evidently being a function among other things of the age or condition of the cuticle. There are lateral wings, though these consist simply of a pair of slightly modified longitudinal striæ.

The conoid neck becomes convex-conoid toward the truncated head, which is not set off in any way. There are six comparatively well amalgamated lips, each of which bears two innervated papillæ, one on the forward surface and somewhat forward pointing, and one on the outer surface and somewhat outward pointing. The anterior of these two papillæ is extended beyond the surface of the lip in the form of a minute seta or innervated papilla, and corresponds to the cephalic seta of other species of Diplogaster. The contour of the lip is not much disturbed by the presence of the posterior papilla, which is sometimes very difficult to see. Close behind the lateral papillæ or setæ there are minute openings in the cuticle, which in character closely simulate the amphids in some other species of Diplogaster, notably those of *D. fictor*. No doubt these are really the outward expression of minute amphids. Distally the lips have thin extensions which can close together over the pharynx in such a fashion that the front of the head is comparatively flat, though the tips of these lips may be recurved and point forward so as to make an exceedingly minute elevation at the middle of the front of the head. The latter has its front surface on the whole very slightly depressed.

The pharynx is about as deep as the front of the head is wide, and bears near its base on the dorsal side a relatively large, rather acute movable conoid tooth or onchus, which reaches about one-third the distance to the lips when the latter are closed, but which is relatively farther forward when the mouth is open. In addition there is a very much smaller submedian projection that undoubtedly may be denominated a rudimentary onchus. When the lips are closed the pharynx is a little wider at the base than anteriorly. At the base of the lips, opposite the posterior circlet of labial papillæ, the width of the pharynx is a little more than one-third that of the corresponding part of the head. Posteriorly, however, the width appears to be nearly three-fifths that of the corresponding portion of the head, at least when the head is viewed in profile. The walls of the pharynx are thin but refractive and fairly well ceratinized. The surface of the dorsal onchus is more highly ceratinized than that of other portions of the pharynx. Both the onchus and the wall of the pharynx have a yellowish or brownish color like that of the spicula. The end of the esophagus receives the base of the pharynx and is at once fully two-thirds as wide as the corresponding portion of the head. It continues to have the same diameter for some distance, then begins to expand and continues to do so to somewhat behind the middle of the neck, where it rather suddenly diminishes in diameter in such a way that it is proper to speak of a median bulb, although the anterior end of this bulb is not very distinctly set off by constriction from the anterior esophageal tube. This bulb contains an elongated valvular apparatus which is about one-third as wide as the bulb itself. This latter is three-fourths as wide as the corresponding portion of the neck. Notwithstanding the rather massive character of this median bulb, the succeeding portion of the esophagus is only about one-fourth as wide as the corresponding portion of the neck. However, it soon begins to widen and forms a somewhat pyriform cardiac bulb three-fourths as wide as the base of the neck. This bulb does not contain any very evident valvular apparatus, though in it there are faint indications of a modification of the esophageal lining. The intestine joins the posterior surface of the cardiac swelling, and at this point is about one-third as wide as the corresponding portion of the body. There is no very distinct cardia. The intestine widens out rather gradually and attains a width at least half as great as that of the body.

The tail end of the female begins to taper from some distance in front of the anus. This latter is slightly raised, especially its broader posterior lip. Behind the anus the tail diminishes somewhat more rapidly for a short distance and thereafter tapers regularly to the hairfine terminus. From the anus the rectum, which is about as long

as the anal body diameter, extends inward and forward. Nothing definite is known with regard to the lateral fields.

From the well-developed, slightly depressed vulva the vagina leads inward at right angles to the ventral surface halfway across the body, where it joins the two symmetrically placed uteri. The internal female organs are double and reflexed, and the ovaries, which are rather narrow and packed with small ova arranged irregularly, reach back to the vulva or even beyond. The ellipsoidal eggs are about as long as the body is wide and about two-thirds as wide as long. Their shells are smooth and rather thick. Specimens have been seen in which well-developed embryos existed in the eggs contained in the uteri. Other specimens have been found in which two to three dozen embryos had escaped from the eggs and then devoured the whole interior of the mother's body. The excretory pore is located opposite the cardiac swelling.

Male formula. $\frac{.9\ \ 11.\ \ 15.\ \ 'M^{68}.\ \ 89.}{2.2\ \ 5.4\ \ 6.1\ \ 10.\ \ 4.6}$ 0.8 mm. The tail of the male diminishes suddenly in diameter from the raised anus in such fashion that at a distance from the anus not very much greater than the anal body diameter it has a diameter only about one-fourth to one-fifth as great as at the anus. At this point, which is immediately behind the posterior group of male papillæ, the tail begins to taper rather gradually and somewhat uniformly, and continues so to do to the hairfine terminus, though there is at first a very slight increase in the diameter, so that the tail has the appearance of being very slightly constricted just behind the posterior caudal group of male papillæ. There is no spinneret, and there are no caudal glands. The two equal, rather slender, tapering, arcuate, brownish, acute spicula are about one and one-half times as long as the anal body diameter. At their widest part, a little distance behind the cephala, the spicula have a width about one-tenth as great as that of the corresponding portion of the body. From this widest part they taper gently toward the cephalated proximal ends. In the other direction the spicula taper regularly to their acute terminals. The accessory pieces surround the spicula at their distal extremities. The portion of the spiculum surrounded by the accessory piece constitutes about one-sixth of the length of the former. Extending backward from this encircling part of the accessory piece is a median arcuate portion arranged nearly parallel to the spicula and having its proximal end somewhat cephalated. The entire length of the accessory piece, including this median dorsal portion, is about one-third that of the spicula. Like the spicula the accessory pieces are brownish in color.

The hemispherical-conoid innervated supplementary male organs are located as follows: In front of the anus three pairs, two of which are ventrally submedian and one sublateral; the sublateral pair is nearly opposite the middle of the spicula, and is on nearly the same zone as the posterior of the two ventrally submedian pairs; the anterior submedian pair is a little in front of the proximal ends of the spicula. Behind the anus the papillæ are arranged as follows: One pair subventral or ventrally submedian immediately behind the anus, two pairs sublateral, and three closely approximated pairs of small size, subventral. This latter group of three pairs is slightly farther behind the anus than the foremost preanal pair is in front of it. The three pairs do not appear to be uniform in structure, the two anterior appearing to be mere innervations, while the posterior one is a distinctly raised innervated papilla like the preanal ones. The posterior of the two pairs of sublateral postanal papillæ is a trifle in front of the group of three just mentioned, while the anterior is about halfway between the group of three and the anus. The anterior border of the anus constitutes a sort of rudimentary flap with an innervation. The testis is single and rather broad and tubular. It extends forward and is reflexed a short distance behind the base of the neck. The reflexed narrower part of the testis is about twice as long as the corresponding body diameter.

Habitat: Manhattan, Kans. Found feeding on grasshopper eggs after the eggs had been deposited in the ground.

The eggs of *Diplogaster aerivora*, which are elliptical in shape, averaged about 0.062 mm. in length and 0.0335 mm. in diameter. When freshly deposited, they were dark brown in color, but became transparent as the embryo developed. Segmentation often began before the eggs were deposited and the succeeding cell divisions could (fig. 3, *F–M*) be readily followed throughout. The eggs were numerous and could be found lying close together in groups of from about 6 to 30. The eggs hatched in about 18 hours from the time segmentation was first noticed. Toward the end of the egg stage the living worm (fig. 3, *M*) could be plainly seen moving about within the egg wall. These movements became more active until the worm finally ruptured the wall and escaped.

At the time of hatching, the young nematodes (fig. 3, *B*) of this species averaged 0.2145 mm. in length. At this stage the sex organs could not be distinguished, because of their poor development. In water cultures the worms grew very rapidly and reached maturity in three to four days. The females matured slightly in advance of the males (fig. 2, *J*). *D. aerivora* never exceeded 0.5 mm. in length nor completed its life cycle while within the termite (fig. 3, *A*). The nematodes remained in the termite in this form for an indefinite length of time, but upon emerging into moist soil they matured in about two days.

Although molting occurred in this species as in *D. labiata*, it was much more difficult to observe; and, while it was not observed more than once in any individual, it is probable that more molts did occur. Molting required less time in *D. aerivora* than in *D. labiata*, and the posterior end of the nematode remained free throughout the process.

In the older water cultures the adults became so numerous that they appeared as a living mass to the naked eye. The females, which were much larger than the males, averaged 0.99 mm. in length and 0.067 mm. in diameter, while the males averaged 0.75 mm. in length and 0.046 mm. in diameter. When free in moist soil, the worms became even larger; the females (fig. 3, *D*) averaged 1.632 mm. in length and 0.1192 mm. in diameter, and the males (fig. 3, *E*) averaged 1.1425 mm. in length and 0.0724 mm. in diameter.

When reared in water cultures, the females appeared darker than the males, but when found in the soil both sexes appeared pearly white. The alimentary canal of the female, like that of *D. labiata*, was spiral, while that of the male was straight. The posterior end of the female's body tapered into a long, threadlike process, but in the male this process was shorter and its body ended in an abrupt hook.

Process of mating.—The process of mating in *D. aerivora* (fig. 2, *L*) was much the same as in *D. labiata*. The male clasped the female slightly back of the middle of the body, so that its anal opening was in direct apposition to the genital pore of the female. In mating, the posterior end of the male usually completely circled the body of the female, although exceptions occurred. Although many instances of mating

were observed, none lasted over 4½ minutes. As the mating neared completion, the female became more active and broke free.

Relation and economy of the sexes.—Both males and females mated repeatedly with different individuals. A single female was observed to mate with 7 different males, and during this time laid a total of 317 fertile and 14 infertile eggs. The length of time from the first to the last mating was 13 days. The greatest number of fertile eggs produced from a single mating by any individual under observation was 125, but the average number was 52.63. A single male was successfully mated with 10 different females, the latter depositing 624 fertile eggs. The total time which elapsed during these 10 matings was 19 days.

Time and method of oviposition.—A single instance was observed of a female depositing a fertile egg 30 minutes after mating, although from one to two hours are usually required. The eggs developed in the ovaries in large numbers and were rapidly discharged through the genital pore. With age the females became very sluggish and did not appear to be able to discharge their eggs; consequently these eggs hatched within the body of their parent, where they fed on her internal organs. Usually they were unable to escape, although instances were observed where they escaped through the genital pore of the mother (fig. 2, *K*).

Proportion of sexes.—Three hundred specimens were examined, and of these 138 were males and 162 were females. In all cultures the females seemed to be more abundant.

Habits.—These nematodes were found parasitic in the heads of *Leucotermes lucifugus*, where under natural conditions the number varied from 0 to about 75. Where heavy infestation occurred, the termites became sluggish and often died. These worms were usually more numerous in the immediate region of the mouth parts of *Leucotermes lucifugus*, although it was not uncommon to find them in the upper part of the cavity of the head. A great many termites were dissected, and in no case were nematodes found in the abdomen. In infested colonies nematodes were often seen in the surrounding soil. These usually were found in masses, feeding upon the bodies of dead termites or other available decaying matter. Specimens of *D. aerivora* placed in water cultures were found to flourish in the same food that was used for *D. labiata*. It was necessary to feed these nematodes each day, for without food they died in a very short time. As in *D. labiata*, the presence of food appeared to stimulate copulation and consequently caused an increase in oviposition.

So far as could be determined, these nematodes showed no preference to either day or night in mating, oviposition, or other habits.

Length of active breeding stage.—The active breeding life of the female extended over a period of about 13 days, while that of the male was about 19 days. The complete life cycle of *D. aerivora* required from four to five days. As the individuals of this species which were

examined had no hibernation stage, their life cycle was continually repeated under favorable conditions. Insufficient moisture and lack of suitable food seriously interfered with the development of these nematodes.

A series of experiments was carried on to ascertain whether it is possible to introduce these parasites into *Leucotermes lucifugus*. Good cultures of nematodes were obtained in moist soil, into which specimens of *L. lucifugus* were placed. After two days a number of these termites were dissected, and it was found that there was an average of 22.9 nematodes in each head. In three days this average rose to 32.9 and in four days it was 46.6. In each instance the check count remained the same, being about 3 nematodes per head. After remaining in a similar culture for 12 days, all the termites died and the bodies were found to be literally alive with nematodes.

SUMMARY

(1) The eggs of *Diplogaster labiata* hatched in from 30 to 32 hours, while those of *D. aerivora* hatched in about 18 hours.

(2) The eggs of *D. labiata* were deposited singly, while those of *D. aerivora* were deposited in groups.

(3) More cases of eggs hatching in the body were found in *D. aerivora* than in *D. labiata*.

(4) The eggs of both species developed similarly.

(5) Both species, when reared in water cultures, used the same food, but in nature they had different hosts.

(6) Both species molted, but the process differed in that *D. labiata* fastened its posterior end, while *D. aerivora* did not.

(7) The adults of *D. aerivora* were larger than those of *D. labiata* and required much less time to mature.

(8) In water cultures, the females of both species were more numerous than the males.

(9) Although mating was similar in both species, *D. labiata* required more time for the process.

(10) Individuals of *D. labiata* usually mated but once, while those of *D. aerivora* mated repeatedly.

(11) Neither species in their habits showed any preference to day or night.

(12) The females of *D. aerivora* had a period of oviposition of about 13 days, while in *D. labiata* this period lasted only about 2 days.

(13) In both species adaptable and plentiful food acted as a stimulant to reproduction.

(14) Both species attacked insects, but in different regions of the body, as *D. aerivora* was found in the head while *D. labiata* was found in the intestines.

(15) The life cycle of *D. labiata* required more than twice as much time as did that of *D. aerivora*.

(16) *D. aerivora* was successfully introduced into the termites.